Article

Exogenous Application of Mg, Zn and B Influences Phyto-Nutritional Composition of Leaves and Fruits of Loquat (*Eriobotrya japonica* Lindl.)

Muhammad Moaaz Ali ¹ , Raheel Anwar ² , Muhammad Waleed Shafique ³, Ahmed Fathy Yousef ^{1,4} and Faxing Chen ^{1,*}

¹ College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou 350002, China; muhammadmoaazali@yahoo.com (M.M.A.); ahmedfathy201161@yahoo.com (A.F.Y.)

² Institute of Horticultural Sciences, University of Agriculture, Faisalabad 38040, Punjab, Pakistan; raheelanwar@uaf.edu.pk

³ Department of Agronomy, University of New England, Armidale, NSW 2351, Australia; walirao00@gmail.com

⁴ Department of Horticulture, College of Agriculture, University of Al-Azhar (Branch Assiut), Assiut 71524, Egypt

* Correspondence: cfaxing@126.com

Abstract: This study was conducted to analyze the effect of magnesium (Mg), zinc (Zn) and boron (B) on the level of macronutrients (Na, Mg, K, Ca), micronutrients (Zn, B, Mo, Mn, Fe, Co, Ni, Cu), and heavy metals (Cd, As, Hg, Pb) in loquat leaves and fruit tissues (peel, pulp, and seed) using inductively coupled plasma-mass spectrometry (ICP-MS). Fruits were obtained from a loquat orchard located in Fujian (Yun Xiao/Zhangzhou). The results revealed that the foliar application of Mg increased the concentrations of Mg, K, Ca, Zn, B, Mn, Ni, and Cu in leaves; Mg, Ca, Zn, B, Mo, and Mn in fruit pulp; and Na, K, B, Mo, Co, Ni, and Cu in seeds. Zinc increased Mg, K, Ca, Zn, B, Ni, and Cu in leaves; Fe, Co and Ni in fruit peel; K, B, Mn, Fe, and Co in fruit pulp; and Na and K in seeds. Similarly, B application increased the concentrations of Na, Mg, K, Ca, and B in leaves; Ca and Ni in fruit peel; Na, Mg, Ca, Zn, B, Mn, Fe, and Co in fruit pulp; and Na, K, Ca, Zn, B, Mo, Mn, Co, and Ni in loquat seeds. Overall, mineral concentrations detected in the leaves and fruit tissues of loquats were greatly influenced by the application of Mg, Zn and B. Although heavy metal concentrations in fruit pulp were increased by the foliar application of B, the fruits were under safe limits for human consumption.

Keywords: trace elements; fertilizer; heavy metals; ICP-MS; microwave digestion

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1. Introduction

The loquat (*Eriobotrya japonica* Lindl.) is an evergreen fruit tree originating from the People's Republic of China. It belongs to the family Rosaceae, subfamily Maloideae. It is a rich source of vitamin A, vitamin B6, potassium, magnesium, and dietary fiber [1]. It is a very beautiful orange-colored fruit with a mild sweet and sour taste [2]. It is most widely grown in Japan, Korea, India, Pakistan, and the south-central region of China. It is also grown as an ornamental shrub in California [3]. China is the leading producer and exporter of loquats, and grows it on more than 100,000 hectares. The annual production of loquats in China reaches up to 380,000 tons [4]. More than 30 species of loquat are being grown in temperate and subtropical regions of Asia [5].

Fruits and vegetables are low in energy content but have high nutritive value. They are a rich source of vitamins, fibers, and minerals, which are very essential for the human body [6]. The sufficient intake of fruits and vegetables can remove saturated fats and sugars from the body and enhance the consumption of healthy nutrients and dietary fiber [7]. To avoid certain cardiovascular diseases, a consumption of 440 g fresh fruits and vegetables per day is recommended [8]. Heavy metals (e.g., Hg, As, Cd, and Pb) can influence the functioning

of the nervous system and cause mental illness by affecting the blood circulatory system [9]. Hence, it is very important to investigate the mineral contents of fruits and vegetables.

Fertilizer use is one of the main factors influencing the mineral contents of plants [10–12]. Foliar application of fertilizers is 10–20% more influential than soil application [13]. Magnesium helps the plants by strengthening cell walls, and it improves the uptake of certain nutrients (e.g., N, P, and S) [14]. Magnesium regulates the balance of cations and anions in cells and plays a significant role with K in maintaining cell turgor pressure [15,16]. There is 75% of leaf Mg involved in protein synthesis, and 15–20% has a significant role in chlorophyll pigmentation [17] and acts as a co-factor of a number of enzymes in photosynthetic metabolism [18–20]. Zinc is one of the essential micronutrients, and is crucial for mineral nutrition in plants. Zinc is involved in essential physiological functions in plant metabolism, influencing the growth and productivity of plants [21]. Boron nutrition affects nutrient status in plants by modulating the uptake and translocation of other nutrients [22]. For example, B plays a positive role in uptake of N, P, K, Cu, Zn, and Fe, whereas it negatively interacts with Ca, Mg, and Mn [23–26].

The quadrupole inductively coupled plasma mass spectrometer (ICP-MS) is the most suitable method for determining trace elements in fruits and vegetables, and has prevailed as the most appropriate practice for clinical quantification [27–30]. Element detection through ICP-MS has become a predominant methodology because of its rapidity, determination limits, and the sample quantity needed for analysis [31]. In the present experiment, the contents of macronutrients (Na, Mg, K, Ca), micronutrients (Zn, B, Mo, Mn, Fe, Co, Ni, Cu), and heavy metals (Cd, As, Hg, Pb) were analyzed in different loquat tissues (leaf blade, fruit peel, pulp, and seed) affected by the foliar application of Mg, Zn, and B via inductively coupled plasma-mass spectrometry (ICP-MS).

2. Materials and Methods

2.1. Plant and Soil Sampling

Twenty young loquat trees, growing in the subtropical area of Fujian province (Yun Xiao) (23°57′13.5″ N 117°20′36.0″ E), were selected and marked for periodic monitoring after foliar application of Mg, Zn, and B. Loquat trees were 4–5.5 m tall with canopy diameters of 4–5 m. The plantation distance among trees was 6 × 6 m, approximately. Loquat trees were systematically pruned and thinned, and fertilized with NPK (15:15:15) at concentrations of 5 kg per plant per season for three growing seasons. The experiment contained five treatments including the control (water spray) (T₀), 2% MgSO₄·7H₂O (T₁), 0.1% ZnSO₄·7H₂O (T₂), 0.2% Na₂B₄O₇·10H₂O (T₃), and T₁+T₂+T₃ (1:1:1) (T₄). The foliar application was carried out twice at the full bloom stage with a 3 week interval. Each tree was considered as one replicate of each treatment (four replicates per treatment).

Samples of the leaves and fruits were taken from the south-exposed tree canopy, at about 3–5 m height, at two different fruit maturity stages (i.e., mature green (MG) (40 days after foliar spray) and fully ripe (FR) (70 days after foliar spray). Samples were washed (for 10 s approximately) with a solution of phosphate-free detergent, then with a 0.1 N HCl solution, and finally with distilled water. Leaves, fruit peel, pulp, and seeds were dried at 70 °C, ground, and passed through a 500-µm stainless-steel sieve.

Soil samples were taken from the root zone of each tree, about 2 m from the trunk, and at two depths (0–25 and 25–40 cm) using a spiral auger with a 2.5 cm diameter. Three subsamples around the trunk were taken to make a composite soil sample for each tree. Samples were transported to the laboratory (Institute of Subtropical Fruits, Fujian Agriculture and Forestry University), oven-dried at 40 °C, and crushed to pass through a 2-mm sieve, then ground to <60 µm for element determinations [32]. The soil's structural type was sandy loam with electric conductivity and pH of 0.396 dS m^{−1} and 7.6, respectively. The electric conductivity and pH were recorded with an EC meter (HI-98,304, Hanna Instruments Inc., George Washington, RI, USA) and digital pH meter (Hanna, HI-98,107, Mauritius), respectively. Table 1 shows the level of mineral nutrients and heavy metals in loquat soil and plant tissues determined through ICP-MS before foliar application of Mg, Zn, and B.

Table 1. Mineral nutrients and heavy metal concentrations in loquat soil and plant tissues determined before the foliar application of Mg, Zn, and B.

Nutrients	Soil	Fruit Pulp	Seed	Leaf Blade	Optimum Ranges (Leaves)
Na (mg kg ^{−1})	421.6	73.16	92.83	154.15	61–115
Mg (mg kg ^{−1})	2935.56	2466.47	1350.65	342.27	2700–3800
K (mg kg ^{−1})	21,499.12	5941.8	21,376.39	2823.17	7500–12,000
Ca (mg kg ^{−1})	2740.1	20,412.75	10,651.17	130.77	19,000–28,900
Zn (mg kg ^{−1})	326.56	41.74	22.83	23.19	20–72
B (mg kg ^{−1})	57.23	12.13	18.72	2.45	25–35
Mo (µg kg ^{−1})	738.59	11,232.68	307.3	603.28	-
Mn (mg kg ^{−1})	91.37	283.53	52.34	126.56	15–23
Fe (mg kg ^{−1})	6569.07	68.91	45.96	11,390.06	53–76
Co (µg kg ^{−1})	1360.8	272.23	231.01	4457.18	-
Ni (µg kg ^{−1})	766,314.86	493.58	1690.06	4052.05	-
Cu (mg kg ^{−1})	205.93	2.52	1.81	2.85	5–7
Cd (µg kg ^{−1})	191.75	298.08	187.43	63.16	-
As (µg kg ^{−1})	333.51	30.62	87.24	55,291.61	-
Hg (µg kg ^{−1})	19.59	1.49	1.09	16.13	-
Pb (µg kg ^{−1})	623.18	4432.19	160.23	101,903.25	-

The values in Table 1 show the mean of four replicates. The optimum ranges of mineral nutrients in loquat leaves are given after the findings of Quiñones et al. [33] and Reig et al. [34].

2.2. Chemical Analysis

2.2.1. Instrumentation and Reagents

The inductively coupled plasma mass spectrometer (Agilent7700X, Agilent, Santa Clara, CA, USA) used in this study was combined with a high-efficiency sample introduction desolvating system equipped with a quartz cyclonic spray chamber. Before each analysis, the instrument was tuned for daily performance using Agilent 7700X Sensitivity Detection Limit Solution, Agilent, USA. This is an aqueous multi-element standard solution of Li, Y, Co, Ce, and Tl for consistent sensitivity (⁷Li, ⁵⁹Co, ⁸⁹Y, ¹⁴⁰Ce, ²⁰⁵Tl) and minimum doubly charged and oxide species levels (¹⁴⁰Ce). The concentration of elements in the solution was 10 µg L^{−1}. The internal standard solution (Agilent, USA) had a concentration of 0.01 µg ml^{−1} for each element (²²⁶Re, ¹¹⁵In, ⁷³Ge, ⁴⁵Sc).

Standard solution (1000 µg L^{−1}) for each element (i.e., Na, Mg, K, Ca, Zn, B, Mo, Mn, Fe, Co, Ni, Cu, Cd, As, Hg, and Pb) was purchased from the National Standard Material Research Centre. Nitric acid (HNO₃) used in the experiment was analytically pure, obtained from CNW Technologies GmbH, Germany. The deionized water (1.83 × 10¹⁰ mmhos) was prepared locally. In this experiment, the working parameters of the inductively coupled plasma mass spectrometer were optimized before the test, and the parameter settings are shown in Table 2.

2.2.2. Sample Preparation

After cleaning, glassware and a Pure Teflon (PTFE) digestion tank were soaked in 20% nitric acid (1 + 4, V + V) for more than 12 h, then rinsed with deionized water three times before use. After lyophilizing, 0.5 g of freeze-dried loquat tissue was added into a poly-tetra-fluoroethylene digestion tank. Then, 5 mL of concentrated nitric acid (analytical grade, CNW Technologies GmbH, Dusseldorf, Germany) was added. The digestion tank was gently shaken to completely immerse the sample, and microwave digestion (Mars5, HY–20–164, CEM, Matthews, NC, USA) occurred under the set conditions (Table 3). After microwave digestion, the solution was cooled naturally. The digestion solution was transferred to a 25-mL volumetric flask. The blank sample (control) was prepared simultaneously.

Table 2. The main working parameters of ICP-MS.

Working Parameters	Set Value
Radio frequency power (W)	1550
Plasma gas flow (L min ⁻¹)	15
Carrier gas flow (L min ⁻¹)	1.07
Compensation airflow (L min ⁻¹)	0.00
Spray chamber temperature (°C)	2
Octopole reaction cell mode	Helium
Oxide (%)	<3
Double charge (%)	<1.5
Sampling cone and intercepting cone	Nickel
Sampling depth (mm)	10.0

Table 3. Parameters during the microwave digestion procedure.

Step	Power (W)	Heating Rate (°C/min)	Temperature/°C	Hold Time (/min)
1	1200	12	120	5
2	1200	30	150	5
3	1200	19	190	35

2.2.3. Determination

A single standard series solution was prepared. The mixed standard series solution and sample solution were measured by ICP–MS, and the standard curve method was used for quantification. The mixed internal standard was added to correct for matrix interference and instrument signal drift.

2.3. Statistical Data Analysis

Collected data were analyzed for analysis of variance (ANOVA) and Fisher's least significance difference (LSD) method for the pair-wise comparison of mean values at a 5% significance level using the analytical software package 'Statistix 8.1'. Principal component analysis (PCA) and correlation coefficient values were determined with the Pearson (n) method using XLSTAT Ver. 2019.

3. Results

3.1. Plant Leaves

3.1.1. Macronutrients

Sodium concentration in loquat leaves was reduced after foliar applications of T₁, T₂, and T₄, while it increased with the application of T₃. Leaves of plants treated with T₄ showed the minimum level of sodium (Na) at the mature green stage (56.27 mg kg⁻¹), whereas at the fully ripe stage the minimum Na (74.16 mg kg⁻¹) was recorded in plants treated with T₂. The maximum Na concentration occurred in the plants treated with T₃ at both maturity stages. Regarding Mg, the plants treated with T₁, T₂, T₃ and T₄ showed increased concentrations of Mg as compared to the control, with the maximum Mg was observed in leaves of plants treated with T₁ (MG–2694.88; FR–2745.70 mg kg⁻¹) followed by T₃, T₂, and T₄ at both maturity stages (MG and FR). Similarly, with the applications of T₁, T₂, T₃, and T₄, the K level in loquat leaves increased. The plants that received T₂ showed a maximum K level (15,569.89 mg kg⁻¹) at the mature green stage, whereas at the fully ripe stage the maximum K level (23,508.60 mg kg⁻¹) was depicted in the plants treated with T₃.

The loquat leaves that received foliar applications of T₁, T₂, T₃, and T₄ had enhanced Ca concentrations as compared to the leaves of the untreated plants (T₀). Foliar application of T₁ was proven better as compared to the other treatments in terms of increasing the level of Ca (16,944.45 mg kg⁻¹) at the mature green stage, while at the fully ripe stage the plants treated with T₄ showed their maximum Ca concentration (9950.87 mg kg⁻¹) (Figure 1).

Figure 1. Level of mineral nutrients and heavy metals in the leaves of a loquat as affected by the exogenous application of Mg, Zn, and B. Different letters indicate a significant difference among treatments for each series according to Fisher's least significant difference test at $\alpha = 0.05$. Vertical bars indicate average $\pm$ standard error (four replicates, $p \leq 0.05$). T₀—control; T₁—2% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, T₂—0.1% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, T₃—0.2% $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ and T₄—T₁+T₂+T₃ (1:1:1), MG—mature green stage, FR—fully ripe stage.

3.1.2. Micronutrients

Leaves of the plants that received foliar applications of T₂, T₃, or T₄ showed decreases in the level of Zn as compared to the untreated plants (T₀) (33.26 mg kg^{−1}), while an increase in Zn was observed in plants treated with T₁ (45.03 mg kg^{−1}) at the mature green stage and in plants treated with T₂ (103.45 mg kg^{−1}) at the fully ripe stage. B concentrations increased in the leaves of the plants that received foliar applications of T₁, T₂, T₃, and T₄ as compared to T₀. The maximum level of B (MG—19.83; FR—38.72 mg kg^{−1}) was found in leaves of the plants treated with T₄ followed by T₂, T₁, and T₃ at both maturity stages. In contrast to B, the Mo level decreased with applications of T₁, T₂, T₃, and T₄. The minimum concentration of Mo (MG—70.98; FR—135.49 $\mu\text{g kg}^{-1}$) was exhibited by plants treated with T₃ followed by T₁ and T₂ at both maturity stages.

The maximum concentration of Mn (MG—349.63; FR—119.16 mg kg^{−1}) was observed in plants treated with T₁ at both maturity stages, while other treatments showed varying results. Similar to Mo, Fe concentrations also decreased with foliar applications of T₁, T₂, T₃, and T₄. The maximum concentration of Fe was recorded in the untreated plants (T₀), whereas plants of the other treatments showed non-significant differences among each other with respect to Fe level. Similarly, the plants that received foliar applications of T₁, T₂, T₃, and T₄ exhibited decreases in Co concentration as compared to untreated plants (T₀). Followed by the control (MG—3350.53; FR—2230.43 $\mu\text{g kg}^{-1}$), the maximum concentration

of Co was observed in plants treated with T₂ (1096.18 $\mu\text{g kg}^{-1}$) at the mature green stage, and T₁ (798.54 $\mu\text{g kg}^{-1}$) at the fully ripe stage.

Concentrations of Ni and Cu in leaves of a loquat were observed to increase with the applications of T₁, T₂, T₃, and T₄ at the mature green stage, whereas at the fully ripe stage the concentrations of Ni increased with applications of T₁ and T₂, and decreased with applications of T₃ and T₄. The concentrations of Cu recorded at the fully ripe stage decreased with the foliar applications of T₁, T₃, and T₄, but not with T₂ (Figure 1).

3.1.3. Heavy Metals

There were significant increases in Cd concentrations in loquat leaf blades with applications of T₁, T₂, T₃, and T₄ at the fully ripe stage, while at the mature green stage there were variations among treatments (increasing with T₁ and T₃, decreasing with T₂ and T₄). The maximum As level (MG–58,776.82; FR–42,313 $\mu\text{g kg}^{-1}$) was recorded in the leaves of the untreated plants (T₀) at both maturity stages, while with the applications of T₁, T₂, T₃, and T₄ the As level reduced significantly. Regarding Hg and Pb, concentrations reduced with the applications of T₁, T₂, T₃, and T₄ as compared to T₀. The minimum concentrations of Hg and Pb were recorded in plants treated with T₃ and T₂, respectively, at the mature green stage (1.42 and 390.49 $\mu\text{g kg}^{-1}$, respectively), while at the fully ripe stage, their concentrations in all treated plants remained non-significantly different (Figure 1).

3.2. Fruit Peel

3.2.1. Macronutrients

The Na concentrations in the loquat fruit's peel were reduced by the foliar applications of T₁ and T₃, and increased with the applications of T₂ and T₄ at the mature green stage. The plants treated with T₂ showed their maximum level of Na at the mature green stage (187.66 mg kg^{-1}), whereas, at the fully ripe stage maximum Na (193.44 mg kg^{-1}) was recorded in the plants treated with T₃. Regarding Mg, the plants treated with T₄ exhibited increased concentrations of Mg (1705.86 mg kg^{-1}) as compared to the control (T₀) at the mature green stage, whereas at the fully ripe stage Mg was reduced by the applications of T₁, T₃, and T₄. Similarly, with the application of T₄, K level in the loquat fruit's peel was observed to increase at both maturity stages (MG–12,171.99; FR–26,687.45 mg kg^{-1}). The loquat fruit's peels that received foliar applications of T₁ and T₃ showed enhancement in Ca concentration as compared to the untreated plants (T₀) at the mature green stage. The foliar application of T₃ proved better than to other treatments, showing the maximum level of Ca (15,437.72 mg kg^{-1}) at the mature green stage, while the Ca level was reduced at the fully ripe stage in plants treated with T₁, T₂, and T₃ (Figure 2).

3.2.2. Micronutrients

The plants that received foliar applications showed increased levels of Zn as compared to the untreated plants (T₀) (21.08 mg kg^{-1}), except when treated with T₁ (33.32 mg kg^{-1}) and T₂ (16.97 mg kg^{-1}) at the mature green stage, and T₄ (25.36 mg kg^{-1}) at the fully ripe stage. The foliar applications of T₁, T₂, T₃, and T₄ on the loquat fruit's peel showed that the B concentration was increased in the plants that received a foliar application of T₄ as compared to untreated plants (T₀) at the fully ripe stage, while at the mature green stage the B level was reduced by applications of T₁, T₂, and T₄. In contrast with B, the Mo level decreased with the applications of T₁ (107.09 $\mu\text{g kg}^{-1}$) and T₃ (76.09 $\mu\text{g kg}^{-1}$), as compared to T₀ (129.56 $\mu\text{g kg}^{-1}$) at the mature green stage. Similarly, the plants that received foliar applications showed a decreased level of Mo at the fully ripe stage.

The plants that received the foliar applications showed a non-significant level of Mn as compared to the untreated plants (T₀) (48.80 mg kg^{-1}), except when treated with T₁ (407.34 mg kg^{-1}) and T₂ (64.82 mg kg^{-1}) at the mature green stage, and T₂ (68.34 mg kg^{-1}) at the fully ripe stage. Similarly, the plants treated with T₂ showed a maximum concentration of iron at the mature green stage, whereas at the fully ripe stage the plants that received foliar applications showed a decrease in Fe level as compared to the control. The plants

that received foliar applications of T₂, T₃, and T₄ showed increases in Co concentration as compared to untreated plants (T₀) at both maturity stages. As compared to the control (MG—414.93; FR—481.30 $\mu\text{g kg}^{-1}$), the maximum concentration of Co was observed in the plants treated with T₄ at both maturity stages (MG—1076.99; FR—1274.71 $\mu\text{g kg}^{-1}$). The concentrations of Ni in the loquat fruit's peel increased with applications of T₂ and T₄ at the mature green stage, whereas at the fully ripe stage the concentrations of Ni increased with applications of T₁, T₂, T₃, and T₄. The concentration of Cu recorded at the fully ripe stage decreased with the foliar applications (Figure 2).

Figure 2. Level of mineral nutrients and heavy metals in loquat fruit's peel as affected by the exogenous application of Mg, Zn, and B. Different letters indicate a significant difference among treatments for each series according to Fisher's least significant difference test at $\alpha = 0.05$. Vertical bars indicate average $\pm$ standard error (four replicates, $p \leq 0.05$). T₀—control; T₁—2% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, T₂—0.1% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, T₃—0.2% $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ and T₄—T₁+T₂+T₃ (1:1:1), MG—mature green stage, FR—fully ripe stage.

3.2.3. Heavy Metals

There was a significant increase in Cd concentrations in the loquat fruit's peel with applications of T₁ and T₃ at the mature green stage, while at the fully ripe stage the plants that received foliar applications showed a decrease in Cd level as compared to the control. The maximum As level (MG—123.01; FR—15.52 $\mu\text{g kg}^{-1}$) was recorded in the fruit peels of the plants treated with T₃ at both maturity stages. Regarding Hg, toxicity was significantly increased with applications of T₁ and T₃ at the mature green stage as compared to the control. Similarly, at the fully ripe stage, concentrations of Hg were reduced with foliar applications except in the plants treated with T₄ (13.21 $\mu\text{g kg}^{-1}$). Lead concentrations decreased with foliar applications of T₁, T₂, T₃, and T₄ at both stages. There

was a significant decrease in Pb toxicity resulting from changes in the maturity stage (Figure 2).

3.3. Fruit Pulp

3.3.1. Macronutrients

The Na concentrations in the loquat fruits' pulp were reduced with foliar applications of T₁, T₂, T₃, and T₄ at the mature green stage, while at the fully ripe stage they decreased except in the plants treated with T₃. The plants treated with T₁ showed a minimum level of Na at the mature green stage (75.57 mg kg⁻¹), whereas, at the fully ripe stage, minimum Na (60.65 mg kg⁻¹) was recorded in plants treated with T₂ (Figure 3). Regarding Mg, the plants treated with T₁, T₂, T₃, and T₄ showed variations in concentrations of Mg, where the maximum Mg was observed in the plants treated with T₂ (2594.59 mg kg⁻¹) followed by T₄, T₃, and T₁ at the mature green stage, and T₃ (2543.08 mg kg⁻¹) followed by T₁, T₂, and T₄ at the fully ripe stage. Similarly, through the application of T₁, T₂, T₃, and T₄, the K level in the loquat fruits' pulp was uplifted at the mature green stage. The plants that received T₁ showed their maximum K level (13,135.76 mg kg⁻¹) at the mature green stage, whereas the maximum K level (12,788.59 mg kg⁻¹) was depicted at the fully ripe stage by the plants treated with T₂. The plants that received foliar applications of T₁, T₂, T₃, and T₄ showed enhancement in Ca concentration as compared to the pulp of the untreated fruits (T₀). The foliar application of T₁ was proven to be better than other treatments, showing its maximum level of Ca (MG—10,214.13; FR—20,459.14 mg kg⁻¹) at both maturity stages (Figure 3).

Figure 3. Level of mineral nutrients and heavy metals in loquat fruit pulp as affected by the exogenous application of Mg, Zn, and B. Different letters indicate a significant difference among treatments for each series according to Fisher's least significant difference test at $\alpha = 0.05$. Vertical bars indicate average $\pm$ standard error (four replicates, $p \leq 0.05$). T₀—control; T₁—2% MgSO₄·7H₂O, T₂—0.1% ZnSO₄·7H₂O, T₃—0.2% Na₂B₄O₇·10H₂O and T₄—T₁+T₂+T₃ (1:1:1), MG—mature green stage, FR—fully ripe stage.

3.3.2. Micronutrients

The plants that received foliar applications of T₂ and T₄ showed decreased levels of Zn concentration as compared to the untreated plants (T₀) at the mature green stage, while at the fully ripe stage there were variations among treatments (increased by T₁ and T₃ and decreased by T₂ and T₄). B concentrations increased in the fruit pulp of the plants that received foliar applications of T₁, T₂, T₃, and T₄ as compared to the untreated plants (T₀) at the mature green stage. The maximum level of B (40.62 mg kg⁻¹) was found in the plants treated with T₁ followed by T₂, T₃, and T₄ at the mature green stage, whereas at the fully ripe stage, the maximum value of B concentration was observed in plants treated with T₂. Similarly, Mo level was also increased by application of T₁ and T₃ at the mature green stage, and T₁ and T₂ at the fully ripe stage. The maximum concentration of Mo was seen in the plants treated with T₁ at the mature green stage and T₂ at the fully ripe stage (Figure 3).

The maximum concentration of Mn was observed in plants treated with T₃ (144.96 mg kg⁻¹) at the mature green stage, while at the fully ripe stage the maximum Mn (436.99 mg kg⁻¹) was observed in plants treated with T₁. Similar to Mo, Fe concentrations decreased with foliar applications of T₁, T₂, T₃, and T₄ at the mature green stage. The maximum concentration of Fe was observed in the untreated plants (T₀) at the mature green stage, while the minimum concentration of Fe was observed in plants treated with T₄ at both maturity stages. Similarly, plants that received foliar applications of T₁, T₂, T₃, and T₄ showed increases in Co concentration as compared to the untreated plants (T₀). The maximum concentration of Co was observed in plants treated with T₄ (MG–961.51; FR–1034.41 µg kg⁻¹) at both maturity stages. Concentrations of Ni and Cu in the loquat fruit pulp decreased with applications of T₁, T₂, T₃, and T₄ at both maturity stages. The minimum concentrations of Ni (MG–837.45; FR–486.52 µg kg⁻¹) and Cu (MG–2641.07; FR–2143.71 µg kg⁻¹) were observed in the plants treated with T₁ at both maturity stages (Figure 3).

3.3.3. Heavy Metals

There were significant increases in Cd concentrations in the loquat fruit pulp following applications of T₁ and T₂, and decreases with applications of T₃ and T₄, at both maturity stages. The maximum As level (21.69 µg kg⁻¹) was recorded in the leaves of the untreated plants (T₀) at the mature green stage, whereas at the fully ripe stage the maximum As was observed in the pulp of the fruits treated with T₃. Regarding Hg, toxicity increased with applications of T₁, T₂, T₃, and T₄ as compared to the control (T₀). The maximum concentration was recorded in plants treated with T₂ at the mature green stage (3.21 µg kg⁻¹), while at the fully ripe stage the maximum concentration of Hg was recorded in the plants treated with T₃. Pb concentration was reduced with the foliar application of T₂ at both maturity stages (Figure 3).

3.4. Seed

3.4.1. Macronutrients

Na concentrations were reduced with foliar applications of T₁ and T₃ at the mature green stage, while they increased with foliar applications of T₂ and T₄. Na concentrations were universally reduced at the fully ripe stage, except in the plants treated with T₃. The plants treated with T₃ showed their minimum level of Na at the mature green stage (47.44 mg kg⁻¹), whereas, at fully ripe stage, the minimum Na (73.63 mg kg⁻¹) was recorded in the untreated plants (T₀) (Figure 4). Regarding Mg, the plants treated with T₁, T₂, T₃, and T₄ showed variations in their concentrations of Mg, with the maximum Mg observed in plants treated with T₄ (2959.40 mg kg⁻¹) followed by the control (T₀) and T₂ at the mature green stage, and T₃ (1241.59 mg kg⁻¹) followed by T₀, T₁, T₂, and T₄ at the fully ripe stage. With the application of T₃, K levels in loquat seeds were slightly uplifted at the mature green stage, but decreased with the application of other treatments as compared to the control. Plants that received T₁ showed their maximum K level (22,396.23 mg kg⁻¹) at the fully ripe stage. Loquat seeds indicated enhanced Ca concentrations in the plants that received foliar applications of T₂ and T₄ as compared to the untreated plants (T₀).

The foliar application of T₄ proved better, however, showing its maximum level of Ca (22,752.88 mg kg⁻¹) at the mature green stage, while T₁ showed its maximum level at the fully ripe stage (Figure 4).

Figure 4. Level of mineral nutrients and heavy metals in loquat seeds as affected by exogenous applications of Mg, Zn, and B. Different letters indicate a significant difference among treatments for each series according to Fisher's least significant difference test at $\alpha = 0.05$. Vertical bars indicate average $\pm$ standard error (four replicates, $p \leq 0.05$). T₀—control; T₁—2% MgSO₄·7H₂O, T₂—0.1% ZnSO₄·7H₂O, T₃—0.2% Na₂B₄O₇·10H₂O and T₄—T₁+T₂+T₃ (1:1:1), MG—mature green stage, FR—fully ripe stage.

3.4.2. Micronutrients

The plants that received foliar applications of T₁, T₂, T₃, and T₄ showed decreased levels of Zn as compared to the untreated plants (T₀) at the mature green stage, while at the fully ripe stage there were variations among treatments (increased by T₁ and T₃ and decreased by T₂ and T₄). B concentrations increased in the seeds of the plants that received foliar applications of T₁ and T₃ as compared to the untreated plants (T₀) at both maturity stages. The maximum level of B (19.58 mg kg⁻¹) was found in the plants treated with T₁ followed by T₃, T₂, and T₄ at the mature green stage, whereas at the fully ripe stage, the maximum value of B was observed in the plants treated with T₃. Similarly, the Mo level also increased with applications of T₁ and T₃ at both maturity stages. The maximum concentration of Mo was seen in the plants treated with T₃ at both maturity stages (MG—152.42; FR—235.82 μ g kg⁻¹). The maximum concentration of Mn was observed in the untreated plants (T₀) (402.29 mg kg⁻¹) at the mature green stage, while at the fully ripe stage the maximum Mn (102.43 mg kg⁻¹) was observed in the plants treated with T₁. Iron concentrations decreased with the foliar applications except in the plants treated with T₂ at the mature green stage. The concentrations also decreased with a

change in maturity stage. The plants that received foliar applications of T_1 and T_3 showed increases in Co concentration as compared to the untreated plants (T_0) at the fully ripe stage, while the maximum level of Co ($979.14 \mu\text{g kg}^{-1}$) was shown at the mature green stage by the seeds of those plants that were treated with T_1 . Concentrations of Ni and Cu increased with applications of T_1 and T_4 in loquat seeds at the mature green stage, whereas Ni concentrations increased with applications of T_1 and T_3 at the fully ripe stage and decreased when T_2 and T_4 were applied. Copper concentrations decreased with foliar applications at the fully ripe stage except when T_1 and T_2 were applied (Figure 4).

3.4.3. Heavy Metals

There was a significant increase in the Cd concentration in loquat seeds with an application of T_4 , and a decrease with T_1 and T_3 at the mature green stage. While at the fully ripe stage, the plants that received foliar applications of T_1 and T_3 showed increased Cd as compared to the untreated plants (T_0). The maximum As ($91.11 \mu\text{g kg}^{-1}$) was recorded in the seeds of the untreated plants (T_0) at the mature green stage, whereas the maximum As was observed in the seeds of the plants at the fully ripe stage treated with T_3 ($87.24 \mu\text{g kg}^{-1}$). Regarding Hg, toxicity decreased with applications of T_1 and T_3 up to 1/2-fold and 1/3-fold, respectively, at the mature green stage. Similarly, at the fully ripe stage, the concentrations of Hg were reduced with the foliar applications except in the plants treated with T_3 ($3.14 \mu\text{g kg}^{-1}$). Lead concentrations decreased with foliar applications of T_1 , T_2 , T_3 , and T_4 at both maturity stages. Toxicity was also reduced with a change in maturity stage from mature green to fully ripe except in the plants treated with T_1 (Figure 4).

3.5. Principle Component Analysis

Principal component analysis (PCA) was applied to Mg, Zn, and B treatments (T_0 , T_1 , T_2 , T_3 and T_4) and phyto-nutritional element concentrations in loquat leaves and fruit peel, pulp, and seed (Figure 5). The macro- and micro-nutrients of leaves in the 2nd and 3rd quadrants demonstrated an inverse relationship with T_0 and positive relationships with T_1 , T_2 , T_3 , and T_4 . This suggests that the application of Mg, Zn, and B increased the concentrations of macro- and micro-nutrients except for those near to T_0 (Fe, Mo, and Co). A cluster of heavy metals (As, Hg, and Pb) located in the 1st quadrant was near to T_0 , indicating an association with the untreated plants. In the peel, phyto-nutrients in the 1st quadrant were found increased in the plants treated with T_0 . These elements included Mo, Fe, Cu, and Pb at the mature green stage and Ca, Zn, Mn, Mo, Cu, and Cd at the fully ripe stage, whereas the elements in the 4th quadrant had a direct relationship with T_2 and T_4 . In the case of fruit pulp, T_0 was in the 2nd quadrant, while T_1 and T_3 were in 1st, and T_2 and T_4 were in 3rd quadrant, indicating a significant difference between the treated and untreated plants. In loquat seeds, concentrations of Pb, Mn, Zn, Mg, and Hg increased in untreated plants at the green mature stage (2nd quadrant). The Ni, Co, and Cu concentrations were greatly influenced by the application of T_1 at both maturity stages (1st quadrant). Similarly, the concentrations of Na, K, Ca, Zn, B, Mo, Cd, and Hg were positively influenced by T_3 treatments. Thus, PCA delineated the effects of Mg, Zn, and B treatments on macro-nutrients, micro-nutrients, and heavy metals in the leaves and fruit tissues of loquats.

Figure 5. Principle component analyses (PCAs) comparing Mg, Zn, and B treatments (observations) and concentrations of phyto-nutritional elements in the leaves and fruit tissues of loquats. T₀—control; T₁—2% MgSO₄·7H₂O, T₂—0.1% ZnSO₄·7H₂O, T₃—0.2% Na₂B₄O₇·10H₂O and T₄—T₁+T₂+T₃ (1:1:1), MG—mature green stage, FR—fully ripe stage.

4. Discussion

There were differences in the concentrations of nutrients and heavy metals between the leaves and fruit tissues of loquats observed at two different maturity stages (i.e., mature green and fully ripe; Figures 1–4). Our findings suggest that Na content in loquat leaves and fruit tissues were suppressed with an application of 2% magnesium sulphate at both maturity stages, while it was increased with a foliar application of 0.2% borax, whereas Mg, K, and Ca contents were increased with an application of 2% magnesium sulphate in leaves of loquat. The obtained results regarding the effects of magnesium sulphate on Mg, K, and Ca contents in loquat leaves are in line with the findings of Ahmed and Morsy [35] on Canino apricots; Mostafa et al. [36] on bananas; Fawzi et al. [37] on Le Conte pears and Hanafy Ahmed et al. [38] on Washington navel oranges. It has been reported that magnesium fertilizer improved leaf Mg content of the aforementioned fruit species. Furthermore, an adequate level of magnesium is required for photosynthesis and chlorophyll formation, which produces carbohydrates in leaves and leads to good yields and high fruit quality [39]. The foliar application of magnesium sulphate at 0.5–3.0% and boric acid at 0.20–1.0% has previously significantly raised percentages of K and Mg in the leaves of Canino apricot compared to a control [40]. Another study revealed that the application of magnesium–potassium based fertilizer decreased Mg and Ca level in leaves, and increased it in Fuji apple fruits [41], although no significant effect was observed of Mg application on the leaf Mg content of olive trees cv. Picual [42].

Loquat leaves have high medicinal value and have been used as folk medicines for thousands of years. Cough, inflammation, chronic bronchitis, cancer, and diabetes are treated with the extract of loquat leaves in Chinese folk medicine [43]. Loquat leaves are rich in phenolics and triterpenes [44]. Concerning human health, it is very important to investigate the effect of foliar-supplied nutrients on the phytonutritional compositions of loquat leaves, as the uptake of elements by plants can be associated with the availability of other elements. A foliar application of 0.1% zinc sulphate decreased the concentrations of Na in leaf blades and fruit pulps, but increased its amount in seeds and fruit peels at the green mature stage (Figures 1, 2 and 4). While at the fully ripe stage, concentrations of Na were found to be decreased in loquat leaves and fruit tissues but not their seeds (Figure 4). The decreased level of Na in loquat plants treated with zinc sulphate have been might be due to less uptake of Na by roots [45]. The concentrations of Mg, K, and Ca in loquat leaves increased as the result of the foliar application of 0.1% zinc sulphate (Figure 1), while Mg was found decreased in the seeds (Figure 4) and K and Ca were also decreased in the peel (Figure 2). Our findings are in line with the findings of Aboyeji et al. [46], who stated that the application of zinc at a concentration of 8 kg ha⁻¹ can increase the K concentration, but that increasing the Zn concentration can lead to a decreased level of Ca in groundnut seeds. The increase in K and Mg could be due to the stimulating effect of Zn X K on wheat [47] and Zn X Mg on tung trees [48]. The reduction in Ca content of loquat seeds could be attributable to the antagonistic effect of Zn, which makes Ca unavailable to plants. Kalyanasundaram and Mehta [49] reported a reduced availability of Ca under high-Zn applications.

In a study, seven rootstocks of citrus were compared under a limited supply of boron, and it was found that the concentration of boron decreased in the leaves from 83.6% to 72.7% in all seedlings. Decreases in Ca, K, Mg, and Zn content and increases in Fe and Mn concentrations were observed in the leaf tissues of scion [50]. Our results also suggest that the loquat plants treated with 0.2% borax showed increased concentrations of Na, Mg, K, Ca, and B, with decreased concentrations of Mo, Mn, Fe, Co, Cu, As, Hg, and Pb in leaves (Figure 1). In accordance with our results (Figure 4), application of 900 mL B ha⁻¹ significantly increased concentrations of Mg, K, and Ca in groundnut seeds [46].

The Zn concentration in the leaves significantly increased with the application of 0.1% zinc sulphate as compared to other treatments observed at the fully ripe stage (Figure 1). The foliar application of zinc sulphate significantly increased concentrations of zinc in the leaf tissues of mango, rice, and coffee, respectively [51–53]. The increase in Zn concentration was found at the fully ripe stage rather than the mature green stage, indicating a slow mobility of Zn in leaves (Figure 1). It has previously been reported that Zn has limited mobility in leaves, moving <25 mm from the application point after 24 h [54]. Our results suggest that the overall concentrations of Zn, Fe, and Cu in leaves and fruits were reduced with a change in maturity (from the mature green to the fully ripe stage). These results are supported by the findings of Madejon et al. [32], who reported that the concentrations of Zn, Fe, and Cu in the leaves, fruits, and seeds of oak and olive significantly reduce over time.

Boron is an essential element for plant growth that plays an important role in different metabolic processes (i.e., cell wall development, respiration, lignification, transport of sugar, phenolic metabolism, IAA metabolism, etc.) [55]. Moreover, it has a vital significance with respect to pollen tube development, the production of flower adhesive material, and pollen viability [56]. Boron concentration in the loquat leaves decreased with the application of borax as compared to the untreated plants (Figure 1). In contrast, the leaves of sweet orange treated with boric acid, zinc borate, and calcium borate showed increased concentrations of boron [57]. Boron concentration in the leaves of coconut palm increased in a dose-dependent manner when boron was applied (0–24 g/plant) [58]. The concentration of boron in the fruit tissues of loquat increased with the application of borax (Figures 2–4). Similarly, the boron concentration in grains of wheat [59], rice [60], and sunflower tops [61] also increased with the application of boron in a dose-dependent

manner. The concentration of Ni in the leaves and fruit pulp of loquat reduced with a change in maturity (Figures 1 and 3). These results are supported by the findings of Madejon et al. [32], who reported that the concentrations of Ni in the leaves and fruit pulp of oak significantly reduced over time.

In order to maintain nutrient homeostasis, plants must regulate nutrient uptake and respond to changes in the soil as well as within the plant. Thus, plant species utilize various strategies for the mobilization and uptake of nutrients as well as chelation, the transport between the various cells and organs of the plant, and storage to achieve whole-plant nutrient homeostasis [62,63]. Following penetration of the cuticle, the uptake of solutes into the cell interior depends primarily on the electrochemical concentration gradient from outside into the cell, but also on the plasma membrane permeability coefficient of the molecule and the degree of cell-mediated active uptake. There are various fates of foliar-applied fertilizer nutrients including retention in the cuticle in solution in the lipoidal layer; penetration of the cuticle followed by movement into the aqueous phase of the apoplast and diffusion into the inner leaf structure; penetration of the cuticle followed by movement with the transpiration water into the mesophyll and symplast; and translocation out of the leaf via the petiole [64].

Schroeder and Balassa [65] were the first to identify that fertilizers are implicated in raising heavy metal concentrations in food crops, and since then researchers have been urged to investigate the impact of impurities in fertilizers on the crop uptake of potentially heavy metals. Studies of heavy metal uptake by plants have often revealed their accumulations at a level that is toxic to human health. More than 70% of cadmium intake by humans is sourced from fruits and vegetables [66]. According to the findings of various scientists, cadmium is a mobile element in the soil, whereas it is immobile in plants, which is why it is found abundantly in plant roots [67–69]. The results indicate a significant decrease in cadmium concentration in leaves (Figure 1) and fruits (Figures 2–4) from the mature green to the ripening stage. A reduction in cadmium concentration over time in the leaves of holm oak and the fruit pulp of olives has also been reported [32]. The maximum concentration of cadmium was observed in plants treated with magnesium sulphate as compared to other treatments (Figures 1–3). This might be due to a reduced translocation of cadmium in plant tissues resulting from the application of Mg [70].

Loquat is one of those fruit crops that is highly sensitive to the quality of irrigation water [71]. Arsenic (As) is a heavy metal that is present in the groundwater of many areas of the world and can contaminate fruits through irrigation water [72]. Many studies have reported on the high level of toxicity in fruits, vegetables, and cereal crops irrigated with arsenic-contaminated water [73]. Our findings show that the toxicity of arsenic concentrations in different loquat plant tissues was reduced with the progression from the mature green stage to the fully ripe stage. These results are in agreement with Madejon et al. [32], who reported a reduction in concentration of As with the passage of time in the leaves and fruits of olives and holm oak. Overall, results suggested that Mg, Zn, and B improved the nutritional composition of loquat leaves and fruit tissues and alleviated the toxicity of heavy metals.

5. Conclusions

Concerning human health, it is very important to investigate the effect of foliar-supplied nutrients on heavy metal accumulation in fruits, as the uptake of elements by plants can be associated with the availability of other elements. Therefore, this study was conducted to confirm the concentrations and availabilities of different phytonutrients in loquat leaves and fruit tissues under the influence of the foliar applications of Mg, Zn, and B. Exogenous application of Mg, Zn, and B proved to be successful for improving mineral nutrition in loquat, as evidenced from the increased macro- and micro-nutrients, and the decreased heavy metals in leaves and fruit tissues. There is need to further investigate Mg-, Zn-, and B-modulated molecular mechanisms regulating mineral uptake and accumulation in leaves and fruit tissues.

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